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Histochemical Study of Skeletal Muscle Fibers in Starved Sheep with Special Reference to Targetoid and Degenerative Fibers

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Summary

The muscles of the starved sheep were histochemically investigated in cryostat sections. The fibers that closely resembled the central core or targetoid fiber were found in the muscles of the starved sheep, and occurred exclusively in the C and D fibers which were light in myosin ATPase reaction. Their occurence varied markedly with the individuals and the muscles used. The degenerative fibers that were stained intensely with azocarmine G changed slightly in succinic dehydrogenase and NADH-diaphorase activity, but gave a negative reaction for phosphorylase and glycogen. The internal positioning of the nuclei was observed infrequently in the C, D and E fibers.

Modern histochemical techniques revealed readily a heterogeneity of skeletal muscle fibers in many vertebrate (1–4), and gave further proof for the presence of affected muscle fibers in tenotomy and denervation, and in human muscle diseases (4–7). In these affected muscles central core (4) or targetoid fibers (5) and target fibers (6, 7) were observed in reactions for NADH-diaphorase and phosphorylase and for myosin ATPase, respectively. In the reactions for cross section, the central core or targetoid fiber has two broad zones, whereas the target fiber has three broad concentric zones. Also, it has been shown that both the targetoid fiber and the target fiber occur virtually exclusively in type I fibers (4, 5).

In the starved sheep, muscle fibers reduced in size owing to disappearance of contractile materials and showed a disorder of pattern of myofibril distribution (8, 9). Moreover, the degenerative or necrotic fibers occurred and the disappearance of the fibers was observed (9). Histochemical studies showed that in the normal sheep the muscle fibers were classified into five types (A, B, C, D, and E) in connection with the relative activity of several enzymes (10–12), and that in starvation the fibers reduced slightly to moderately in succinic dehydrogenase (SDH) activity and markedly in phosphorylase activity and glycogen content (13). However, the histochemical changes varied strikingly with the fiber types.

The objective of the present study was to make additional observations on the histochemical alteration in affected fibers in starvation. Special attention was paid to the occurrence of targetoid fiber and the relationship of the degenerative fiber to oxidative enzyme and phosphorylase activity.

Materials and Methods

Muscle samples were obtained from the same sheep as in the previous study (13). At slaughter, the samples were immediately separated from the belly of the following muscles; *M. serratus ventralis*, *M. supraspinatus*, *M. infraspinatus*, *M. longissimus thoracis* (*M. longissimus dorsi*), *M. semitendinosus*, and *M. masseter*. Experiments on the starvation and the body weight loss of four starved sheep were given in the previous paper (13). Glycogen, lipid, SDH, NADH-diaphorase, two types of ATPase, and phosphorylase were demonstrated histochemically in the same manner employed in the previous study (10). Some of the cryostat sections were fixed by 10% neutral buffered formalin for a few days and used for Heidenhain's azocarmine-aniline blue stain and hematoxylin and eosin stain.

Results

Disorder of distribution pattern of diformazan granules: In SDH and NADH-diaphorase reaction, diformazan deposits of the A and B fibers of the normal muscles gave a granular appearance (10–12). In starvation, diformazan deposits of some A and B fibers gave a fibrous or streak appearance (Fig. 1). In the C fibers, the stellate or open-network pattern of diformazan became much finer than that in the normal C fibers, or changed into a granular or streak pattern. Most of the D fibers showed the same, reticular pattern of diformazan as the normal D fibers. In a few D fibers the reticular pattern of diformazan appeared to change into fine granules, although the fine granules appeared to make up a reticular pattern by a bead-like arrangement. The disorder of distribution pattern of diformazan was seldom observed in the E fibers.

Local disappearance in the activity of SDH and NADH-diaphorase in muscle fibers: In the reactions to SDH and NADH-diaphorase, the diformazan granules disappeared partially and roundly beneath the sarcolemma or in the interior of the fibers (Figs. 2, 9, and 10). This alteration occurred exclusively in the C and D fibers which were light in myosin ATPase reaction. This fiber resembled closely the central core or targetoid fibers (4, 5), and hence is refered to as targetoid fibers in the present paper. The occurrence of the targetoid fibers varied strikingly with the individuals and the muscles observed (Table 1). It was generally greater in the D fiber than in the C fiber.

The targetoid fibers disappeared partially in the activities of mitochondrial ATPase and phosphorylase and in glycogen and mitochondria, but showed no alteration in eosin stain (Figs. 2–12). The extent of the disappearance was greater

TABLE 1. *Occurrence of targetoid fibers* (%)

No. of individuals	1		2		3		4	
Fiber type	C	D	C	D	C	D	C	D
M. serratus ventralis	0	0	9.5	35.7	8.3	43.5	2.3	4.7
M. supraspinatus	0	0	0	0	0	0.8	19.2	76.9
M. infraspinatus	20	22.7	0	0.8	0	0	0	24.4
M. longissimus thoracis	0	—	0	—	0	—	27.8	—
M. semitendinosus	0	—	0	—	0	—	0.7	—

in SDH reaction than in NADH-diaphorase reaction, and smaller in the reactions for phosphorylase and glycogen. The extent of disappearance of NADH-diaphorase activity was generally similar to that of the sudanophilic materials (Figs. 10 and 11). The partial, negative reaction for myosin ATPase in targetoid fibers was rarely seen (Fig. 4). Most of the targetoid fibers were unchanged in myosin ATPase activity. In eosin stain, the targetoid fibers showed an obscure myofibrillar pattern, but no vacuolation (Figs. 8 and 12). The target fibers observed by Engel (6) were not found in the muscles of the starved sheep.

Enzyme activity in degenerative fibers: The fibers that showed a degenerative change in starvation were stained intensely with iron hematoxylin and with azocarmine G in Heidenhein's azocarmine-aniline blue stain (9). Sections that were cut by a cryostat and then fixed with formalin were used for the demonstration of the degenerative fibers. The fibers changed slightly in the activity of NADH-diaphorase and mitochondrial and myosin ATPase, but decreased slightly in SDH activity and reacted negatively for phosphorylase and glycogen (Figs. 13–16). In SDH and NADH-diaphorase reaction, a marked disorder of distribution pattern of diformazan was observed in some of the degenerative fibers. Generally, the degenerative fibers were round and larger than the surrounding fibers, but not larger than the normal fibers. The degenerative change occurred regardless of the fiber types.

Internal positioning of nuclei in fibers: The nuclei of the fibers were located beneath the sarcolemma in starvation as in the normal case. However, the nuclei were infrequently observed in inner positions of the fibers in starvation (Fig. 8). The inner nuclei were observed in the C, D and E fibers, but not in the A and B fibers. The internal positioning of the nuclei had no relationship to changes in the enzyme activity in a given fiber.

Discussion

In the A and B fibers, the alteration of granular to fibrous appearance in diformazan seemed to be caused by the oblique cut. The oblique cut resulted probably from the bending or distortion of the fibers (9). The open-network pattern of diformazan in the C fibers became finer. It was presumed that this alteration resulted from the reduction in myofibril diameter due to the loss of

contractile materials (9). In a few D fibers, the changes in the reticular pattern of diformazan seemed to be due to the transformation or redistribution of mitochondria.

In SDH and NADH-diaphorase reaction, the partial disappearance of diformazan in the targetoid fibers seemed to be due to the partial disappearance of mitochondria and sarcoplasmic reticulum, and independent of the disappearance of contractile materials. This is because the disappearance of diformazan agreed with that of sudanophilic materials, and because the targetoid fibers were affected in the sarcoplasm including phosphorylase and glycogen in addition to mitochondria, but not always in the contractile materials.

The targetoid fibers in the present study resembled closely the central core or targetoid fibers in central core disease, human denervation disease (4), and tenotomized and denervative muscles (5). The targetoid fibers occurred only in the C and D fibers in starvation. This is in good agreement with the results that the central core or targetoid fibers occurred virtually exclusively in the type I fiber equivalent to the C and D fibers.

The degenerative fibers increased in number during starvation (9). They were stained intensely with iron hematoxylin and azocarmine G. In the present study, it was demonstrated that the degenerative fibers disappeared in phosphorylase activity and glycogen, but changed slightly in SDH and NADH-diaphorase activity. It was not, therefore, thought that the degenerative fiber was caused by the disappearance or marked reduction in the dehydrogenase activities, and that the disappearance of phosphorylase activity and glycogen in individual fibers was immediately followed by their degenerative change. This is because most of the atrophic fibers without glycogen and phosphorylase activity reflected no degenerative change. However, the disappearance of glycogen and phosphorylase activity may possibly bear a relationship to the occurrence of the degenerative fibers, since they increased in number in the last stage of starvation (9) and their glycogen decreased or disappeared (8, 13).

The histochemical aspects of the degenerative fibers appeared to be similar to those of the giant fibers of Cassens *et al.*, who believed that porcine giant fiber reflected a pathological change (14). The degenerative fiber resembled closely the giant fibers in microscopic profiles, although there was a marked difference in size.

The internal nuclei of the fibers are observed commonly in experimental denervation and tenotomy (15, 16), vitamin E deficiency (17), hereditary, progressive muscular dystrophy in mice (18), and human progressive muscular dystrophy (19). The internal positioning of the nuclei occurred in the earlier stage of denervation and tenotomy (15, 16). Knowlton *et al.* (20) and West and Mason (17) have interpreted that the internal rowing of the nuclei in vitamin E deficiency may represent a reaction of muscle fibers to mild injury.

In the present study, the internal positioning of the nuclei occurred in the C, D and E fibers. The targetoid fiber was observed only in the C and D fibers. These suggested that such changes reflected a reaction of the fibers to the injury caused by starvation, and that the reaction differed with the dark and the light fibers in myosin ATPase stain. Since the fibers having internal nuclei were very few, and the occurrence of the targetoid fibers varied markedly with the individuals and the muscles, it was insufficient to assume that the changes were a common phenomenon in the muscle fibers of starved sheep. However, it was certainly presumed that the internal positioning of the nuclei and the occurrence of the targetoid fiber in the D fibers reflected their reaction to the injury caused by starvation, although they hardly changed in enzyme activities and showed resistance to atrophy (8, 13).

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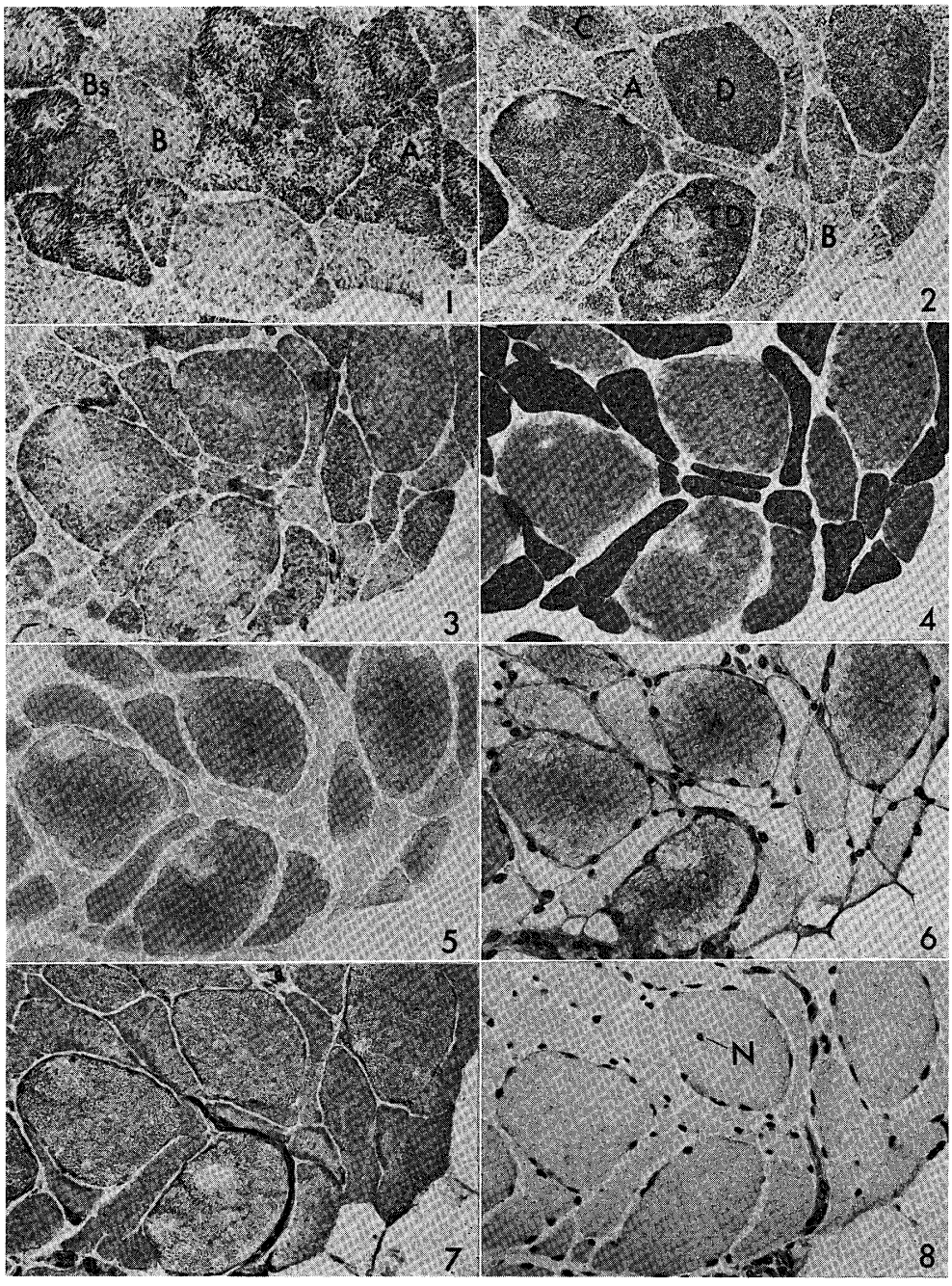
Plate 1

Explanation of Figures

Fig. 1. The *M. semitendinosus* of the starved animal (Sheep No. 4) NADH-diaphorase. In the A and B fibers, diformazan gives a granular (A, B) and a fibrous or streak (As, Bs) appearance. Some of the C fibers (C) show a streak pattern of diformazan. ×300.

Figs. 2 to 8. The *M. supraspinatus* of the starved animal (Sheep No. 4). The same fields of serial cross sections. × 250. Fig. 2. NADH-diaphorase. The D fibers (D) show the reticular pattern of diformazan, but diformazan disappears partially in some D fibers (TD). These fibers (TD) indicates the targetoid fibers. Fig. 3. Mitochondrial ATPase. Fig. 4. Myosin ATPase. Fig. 5. Phosphorylase. Fig. 6. Glycogen (PAS stain). Fig. 7. Sudan black B stain after acetone extraction. Fig. 8. Hematoxylin and eosin stain. The targetoid fibers show no vacuolation. N indicates internal nuclei.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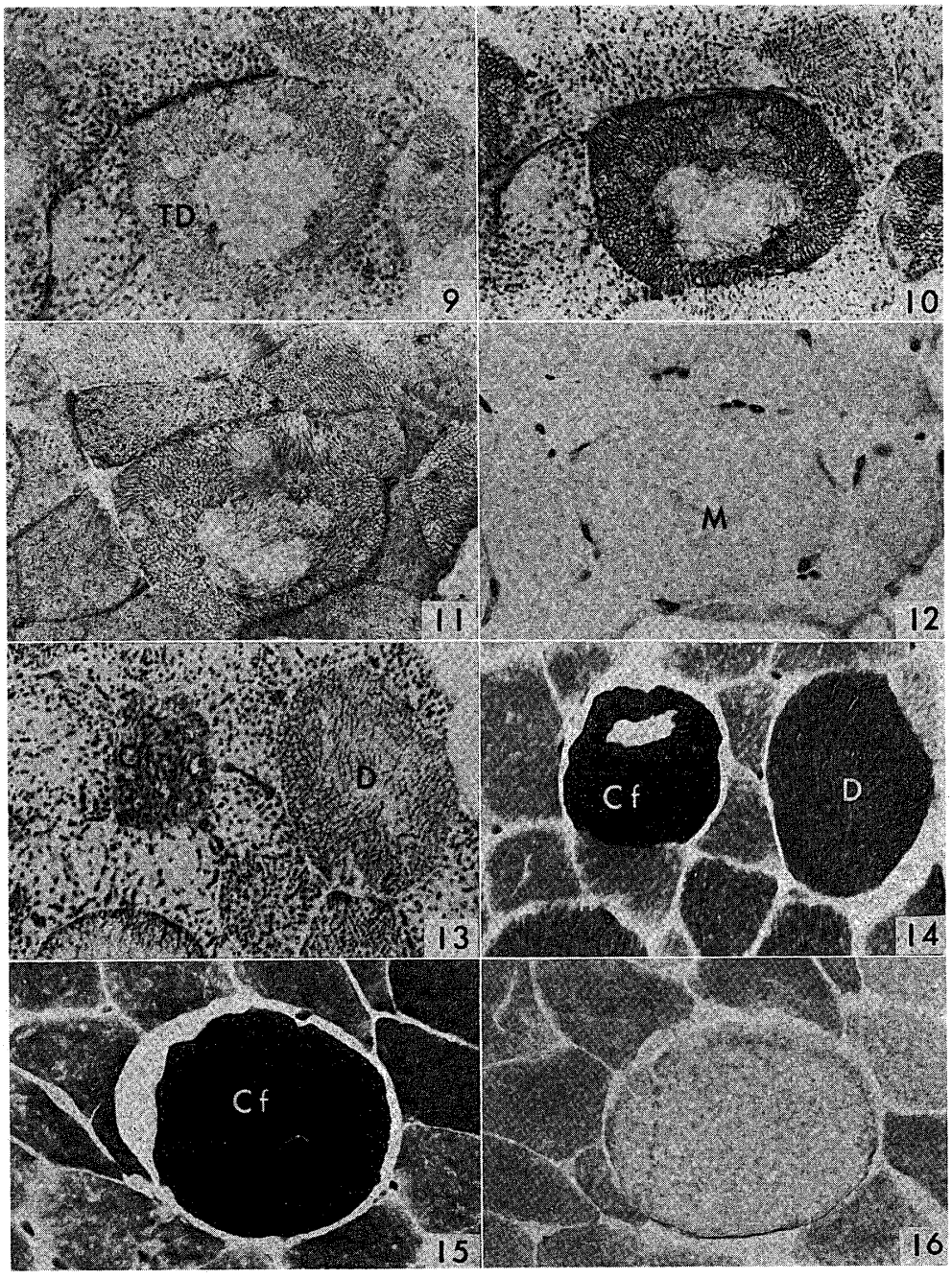
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PLATE 2

Explanation of Figures

FIGS. 9 to 12. The *M. serratus ventralis* of the starved animal (Sheep No. 3). The same fields of serial cross sections. × 360. Fig. 9. Succinic dehydrogenase. Fig. 10. NADH-diaphorase. Fig. 11. Sudan black B stain after acetone extraction. The disappearance of diformazan granules resembles that of sudanophilic materials in the targetoid fiber (TD). Fig. 12. Hematoxylin and eosin stain. The targetoid fiber shows obscuration of myofibrillar pattern (M), but no vacuolation.

FIGS. 13 and 14. The *M. serratus ventralis* of the starved animal (Sheep No. 2). × 340. Fig. 13. NADH-diaphorase. Fig. 14. Heidenhain's azocarmine-aniline blue stain. Degenerative fiber (Cf) reacts strongly for NADH-diaphorase and is stained intensely with azocarmine G. The D fibers (D) are stained more strongly with azocarmine G than other fiber types.

FIGS. 15 and 16. The *M. serratus ventralis* of the starved animal (Sheep No. 3). The same fields of serial cross sections. × 520. Fig. 15. Heidenhain's azocarmine-aniline blue stain. Fig. 16. Phosphorylase. Degenerative fiber (Cf) stained intensely with azocarmine G reacts negatively for phosphorylase.